A (INSERT)   C (REPRESSION CASSETTE)

INSERT DONOR   VECTOR DONOR

B (CLONING VECTOR)   D (SUBCLONING VECTOR)

RECOMBINASE □

A   D

COINTEGRATE

B   C

RECOMBINASE ○

A   C

SUBCLONE=   BYPRODUCT
PRODUCT

D   B

FIG.1

FIG.2

FIG.3

FIG.4

FIG.5

FIG.6
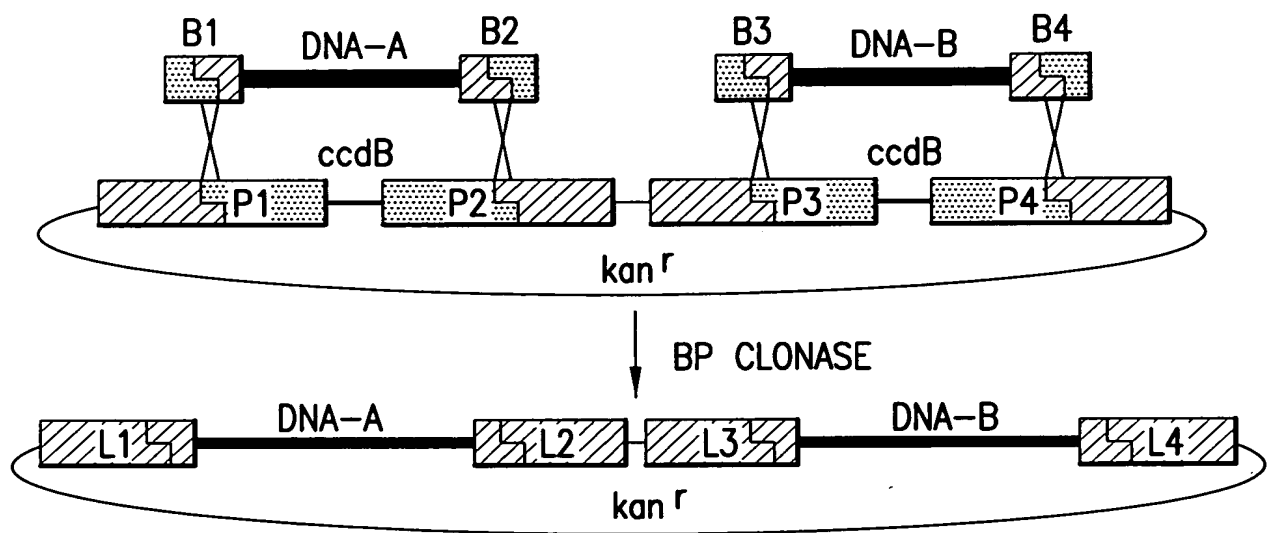
FIG.7

FIG.8

FIG.9

FIG.10

FIG.11

FIG.12

FIG.13

T7
PROMOTER

GUS

attB

TAG

attB

GST

supF

TRANSCRIPTION
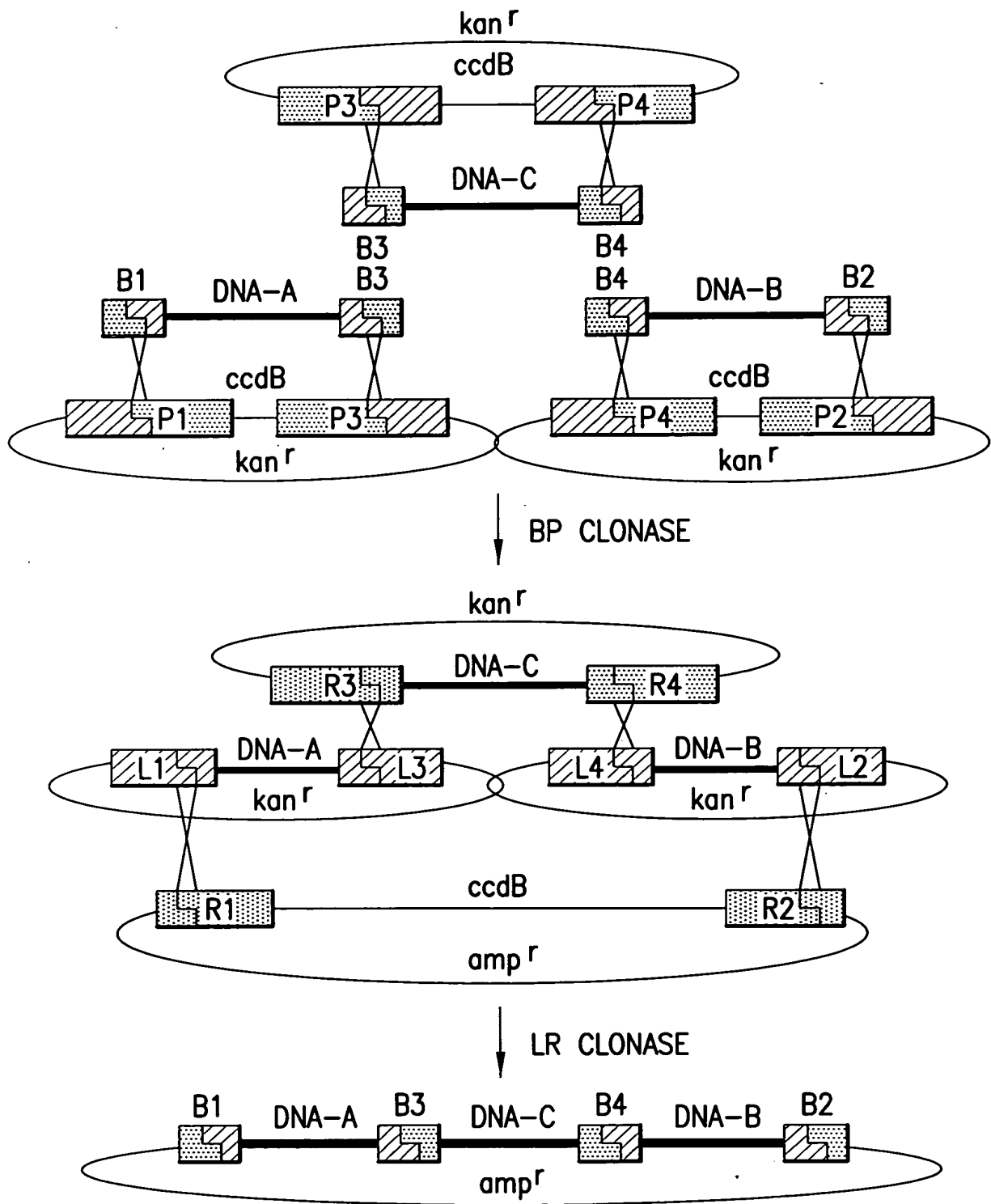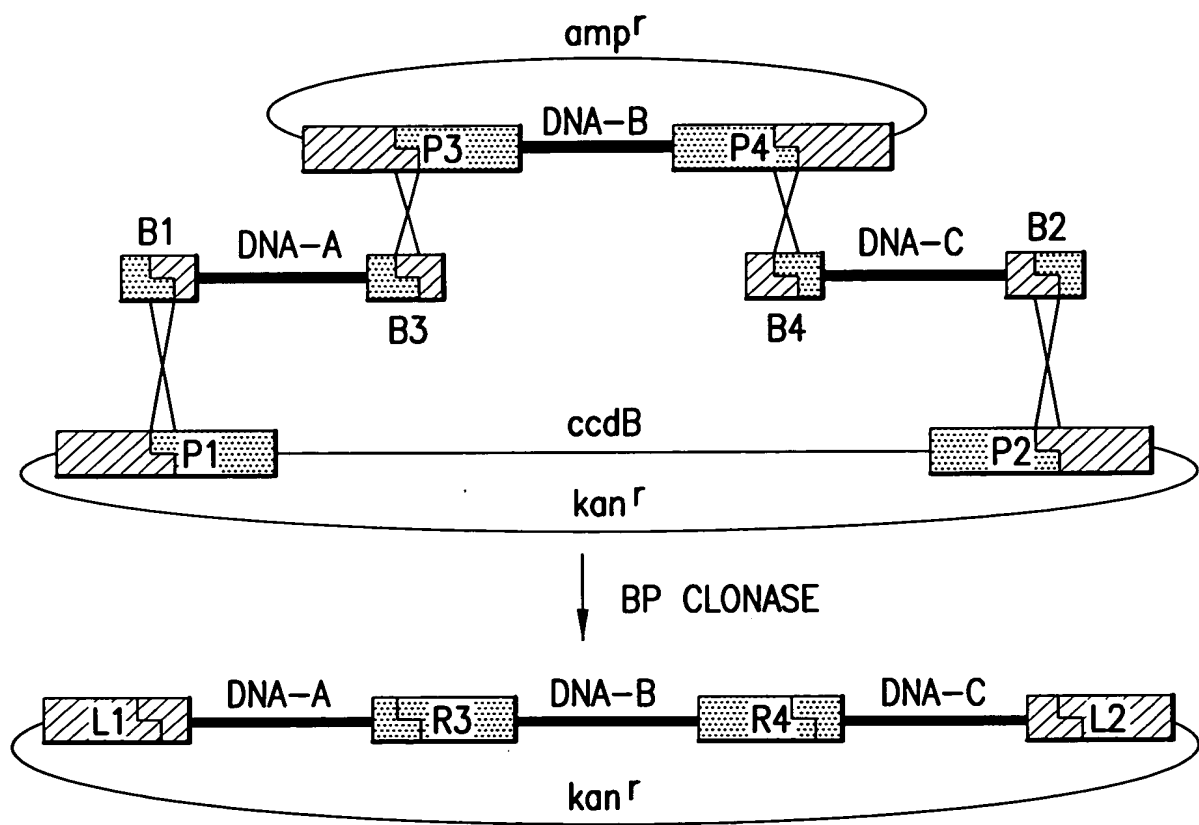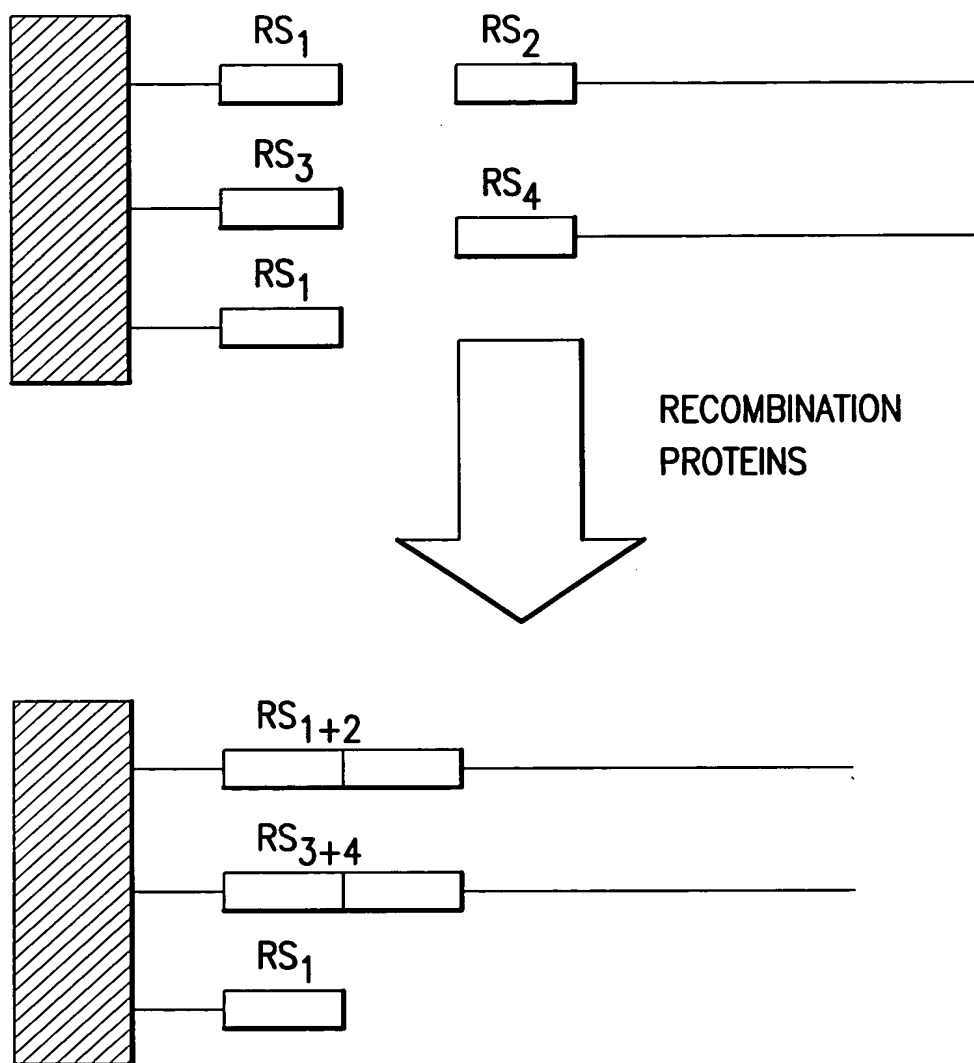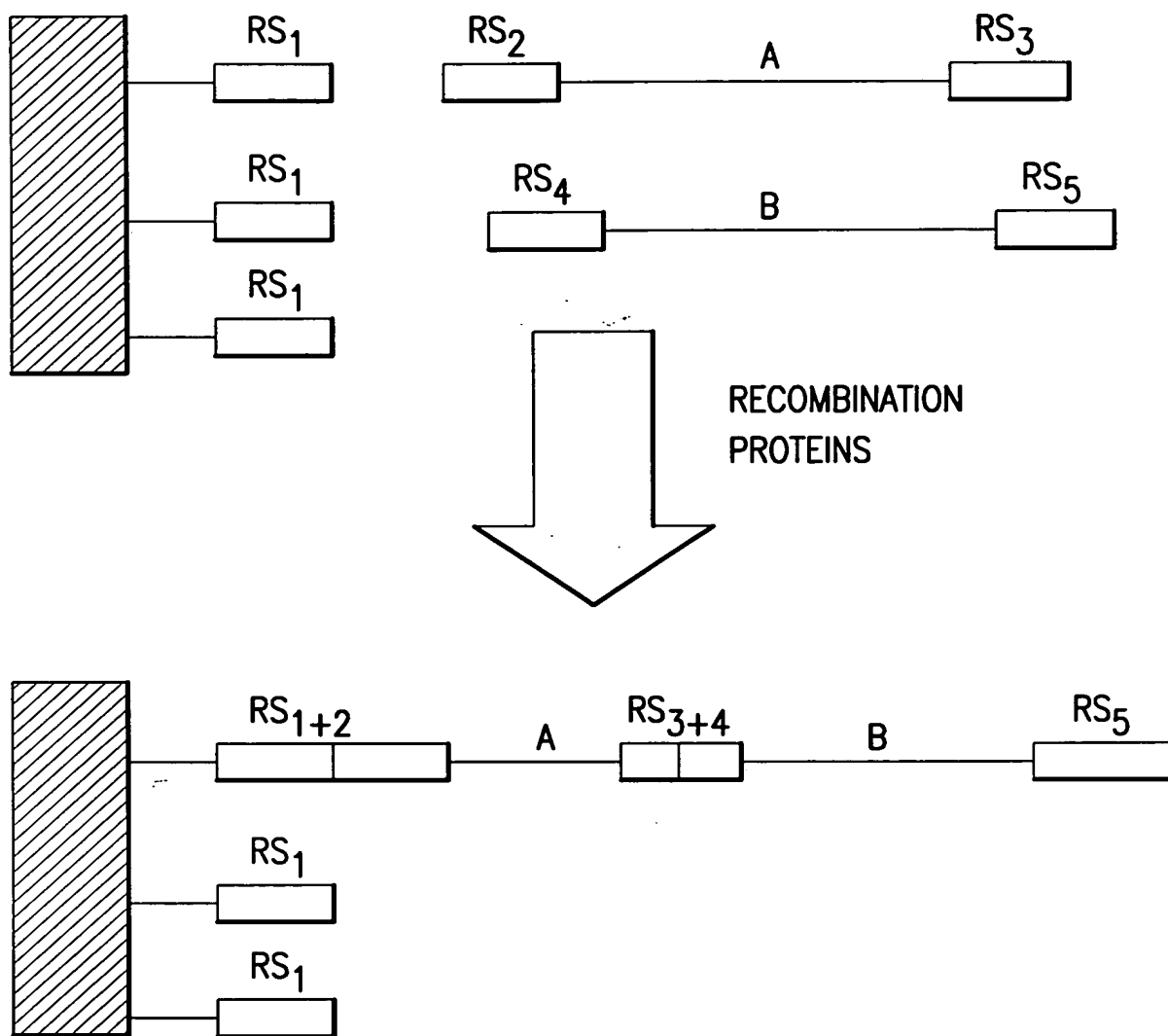TERMINATOR
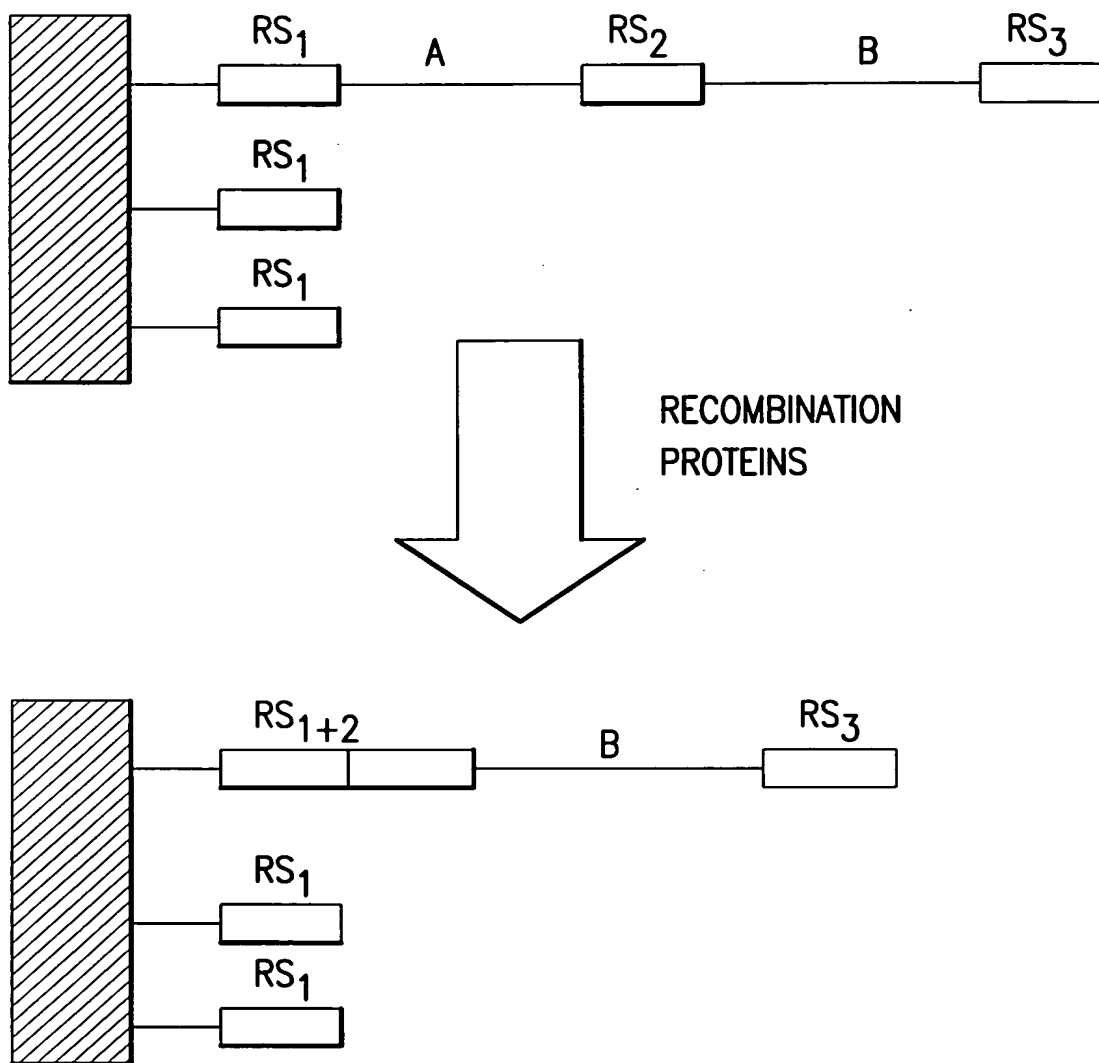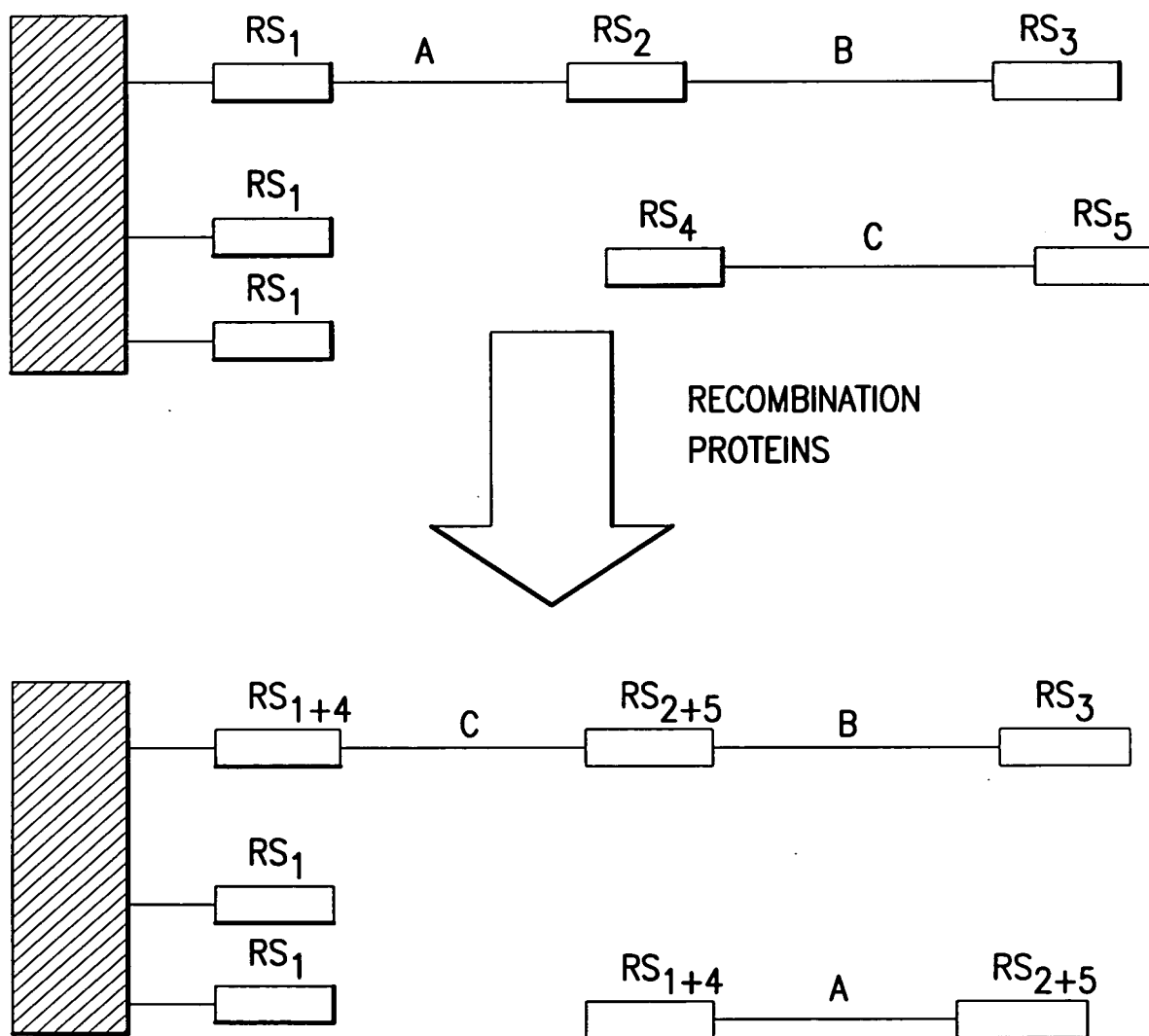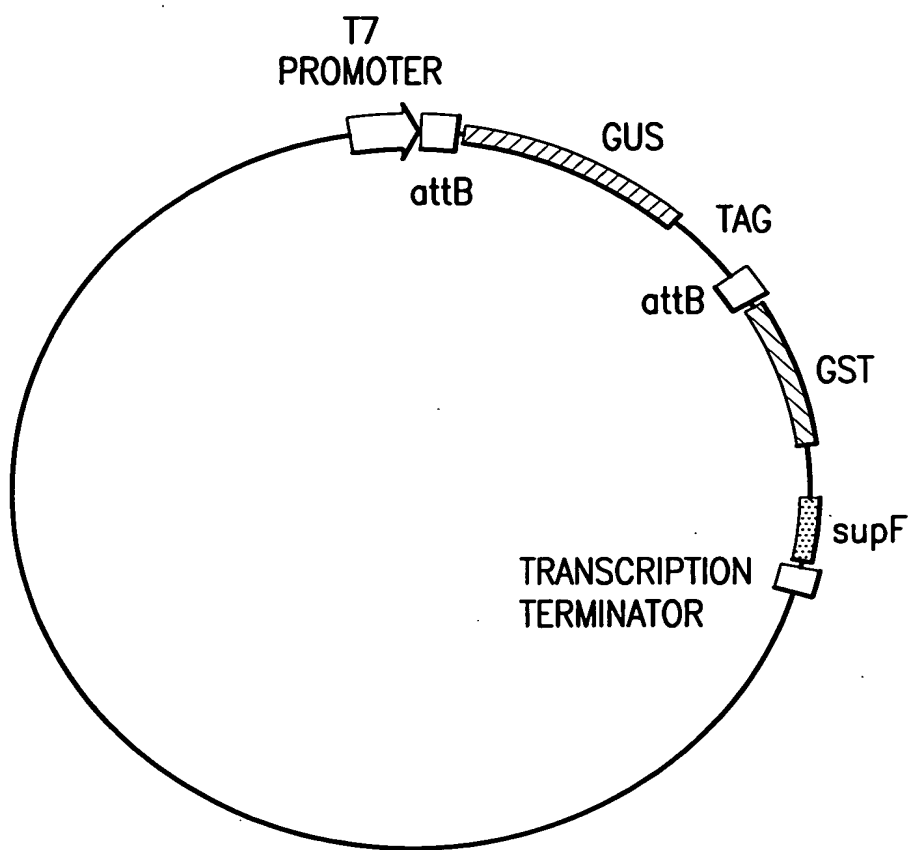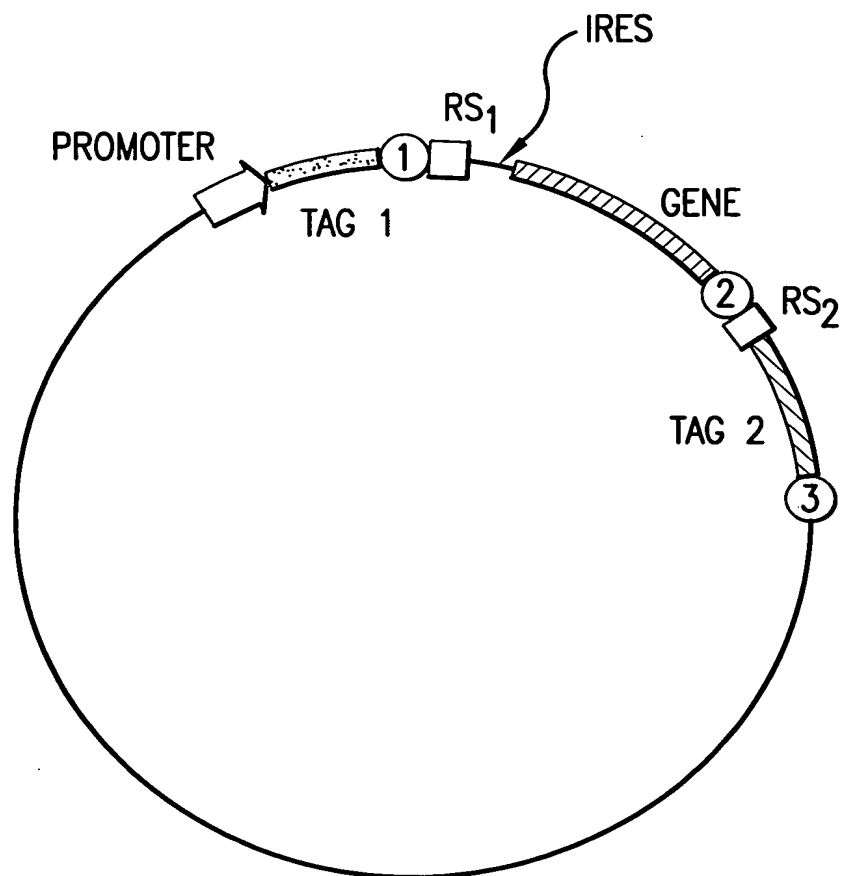
FIG.14A

FIG.14B

FIG.15

FIGURE 16
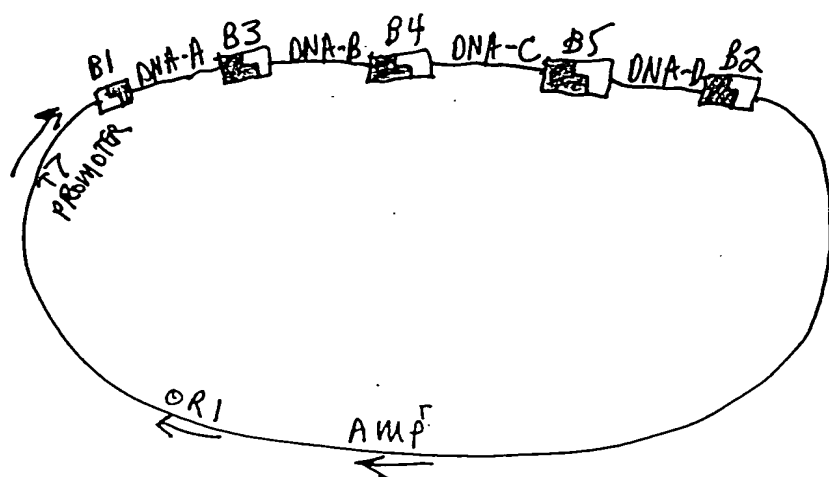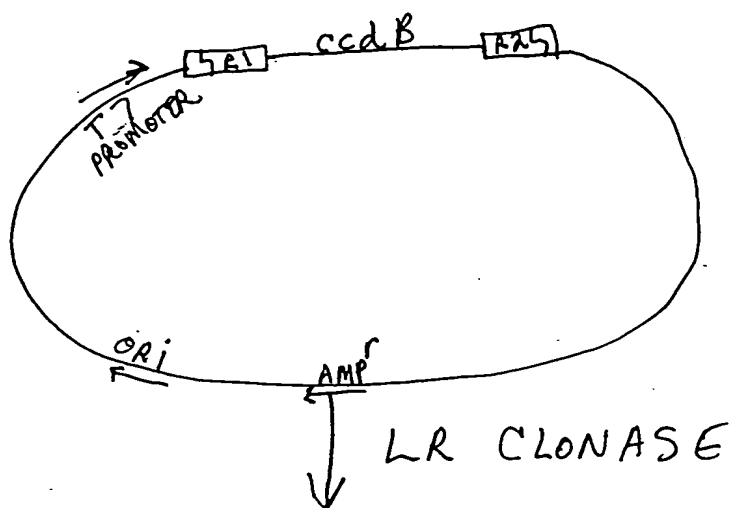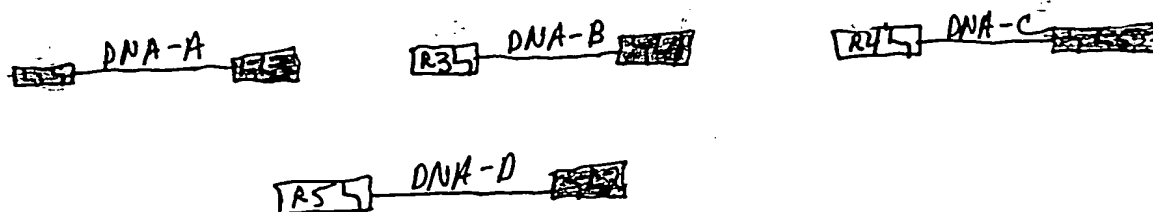
FIGURE 17A

Cloning Light
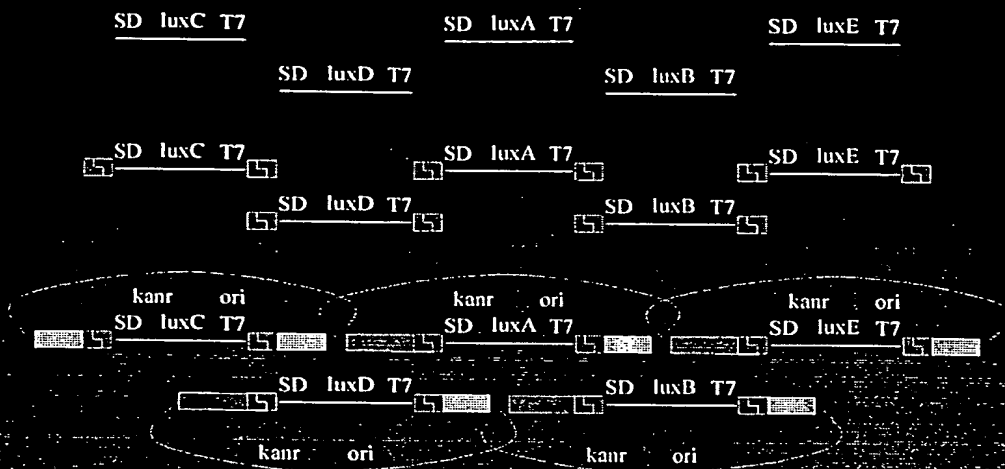
FIGURE 17B

Cloning Light
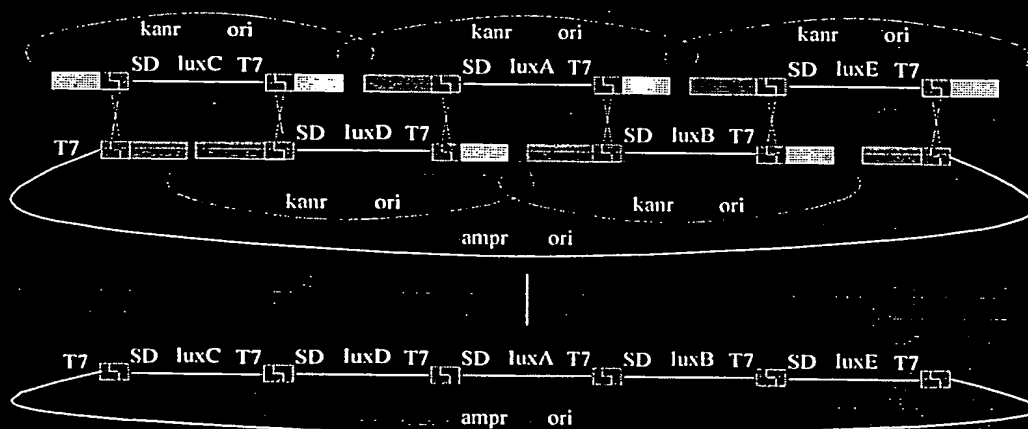
FIGURE 18
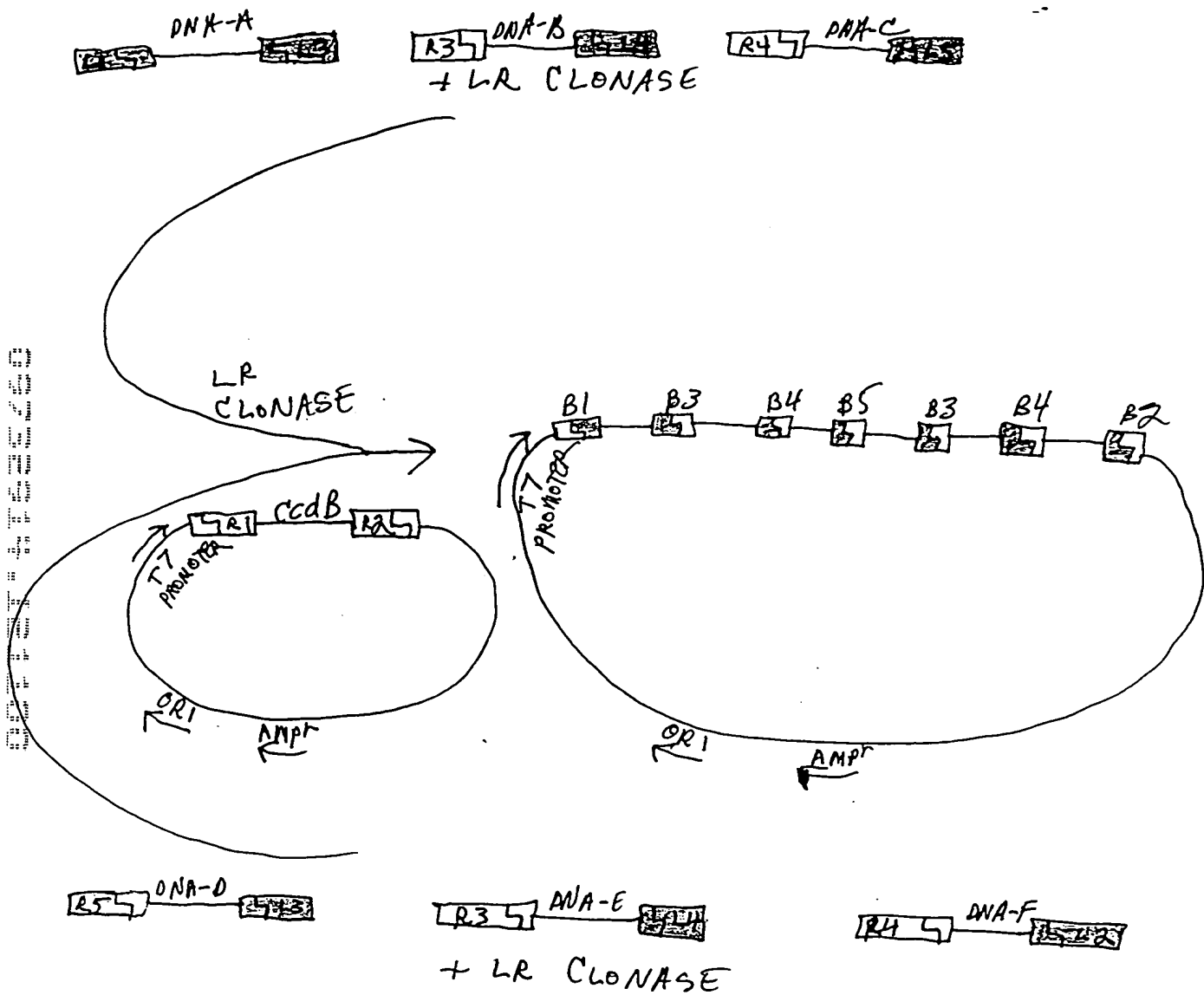
FIGURE 19

FIGURE 20

FIGURE 21

A.

B.

C.

FIGURE 22A

FIGUR 22 B

FIGURE 23

mRNA

——————————————————————————————AAAAAA

↓ Random-primed 1st strand
synthesis

↓ Random-primed PCR

═══ ══ ═ ═══ ══ ═══

↓ Split into n reactions

↓ PCR with primers with random
3' ends, attB sites on 5' ends

$B_i = B_{i\ 1}$ 　　 $B_{(i+1)_R} = B_{i+2}$ 　　 $B_{(i+2)_R} = B_{i+3}$ 　　 $B_{(n)_R} = B_{n+1}$

Size fractionate, e.g. by gel purification,
to 50 - 60 bp

↓ Clone into Entry vectors

↓ Pool Entry clones

↓ Clone concatemers

seq. primer → $B_i = B_{i+1} = B_{i+2} = B_{i+3} = B_{i+4} = \cdots B_n = B_{n+1}$

Vector

↓

Sequence concatemer
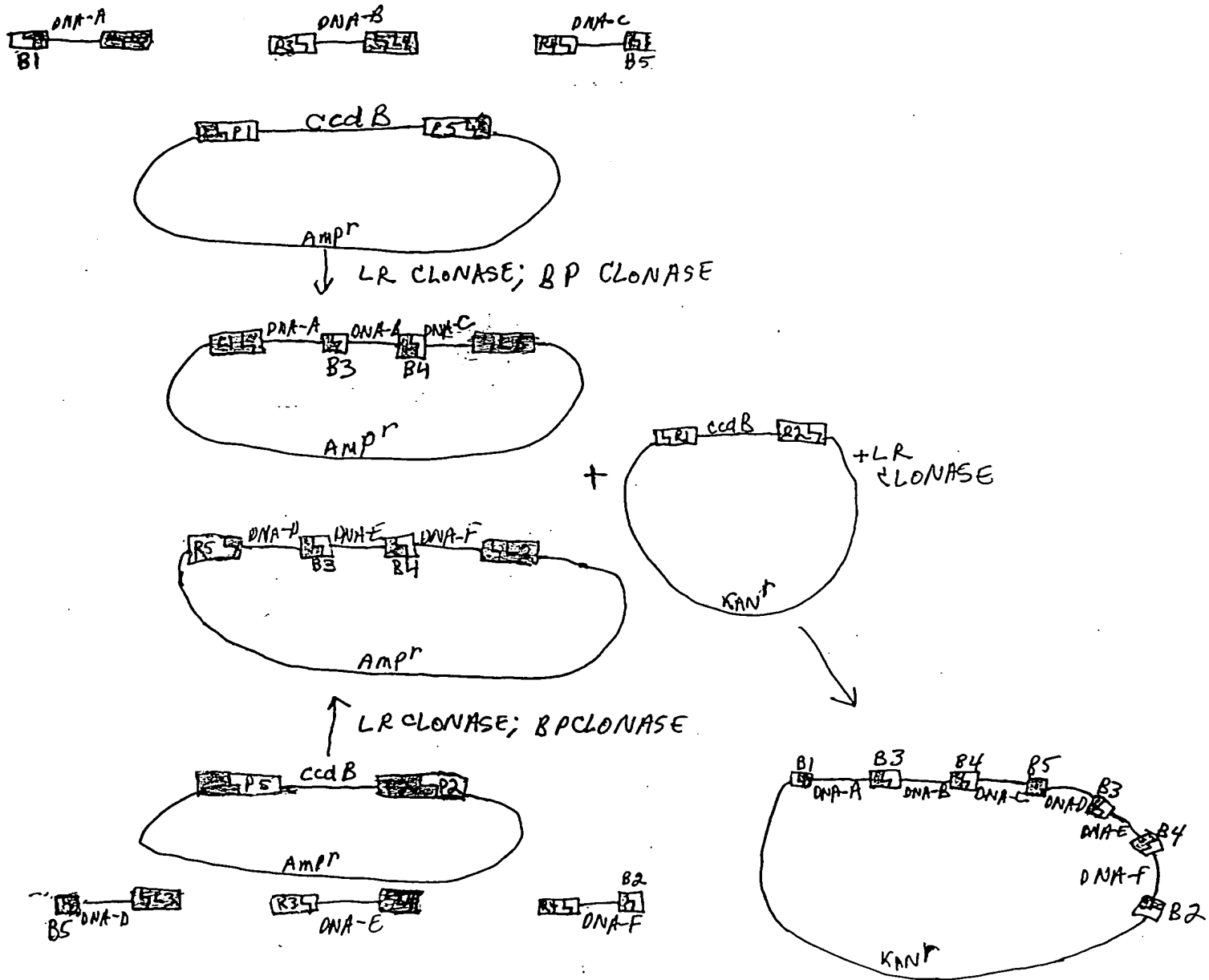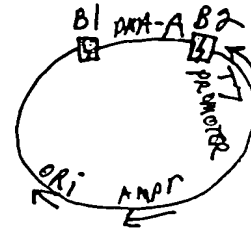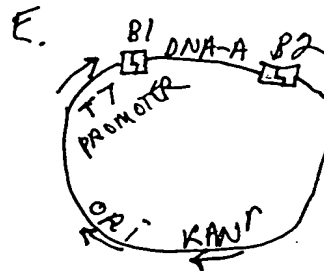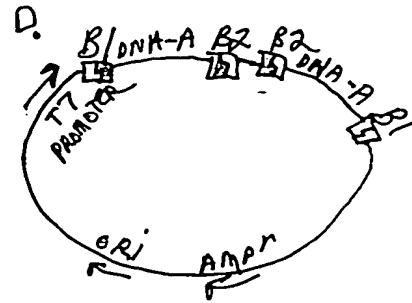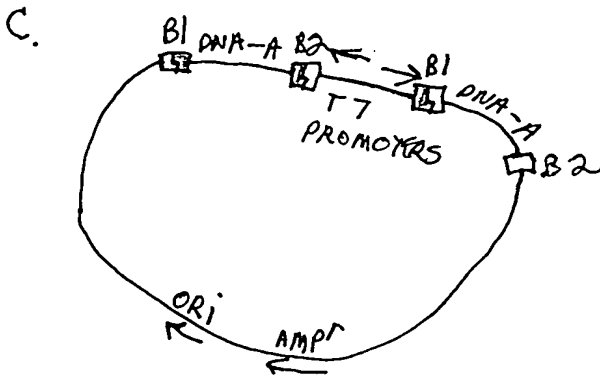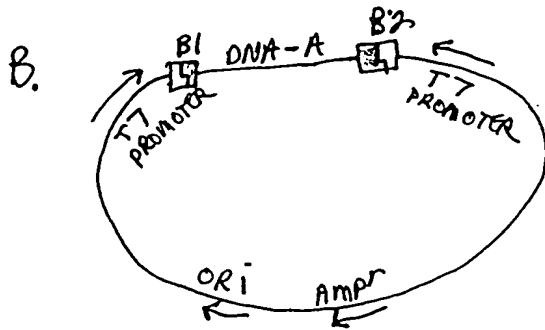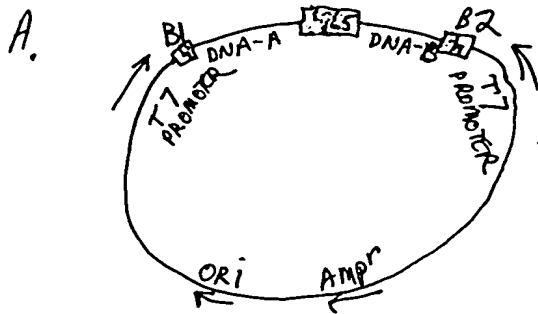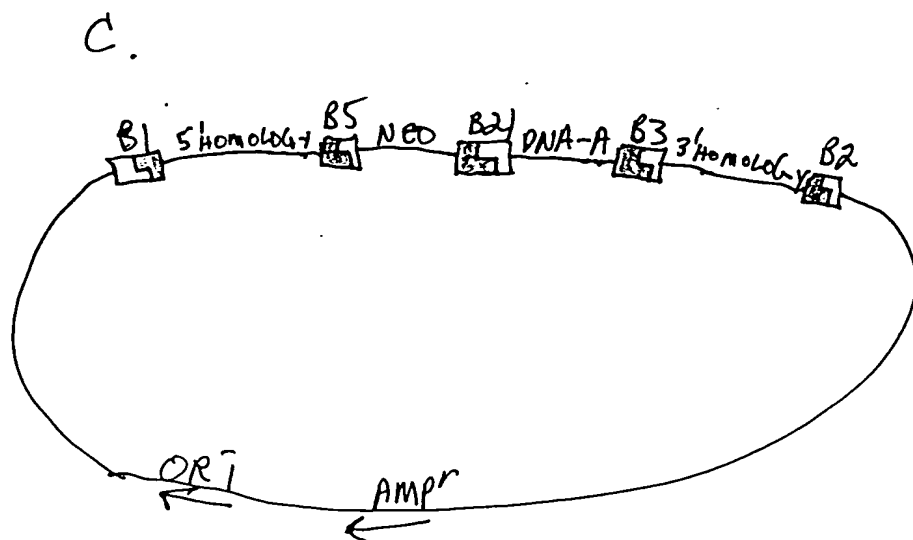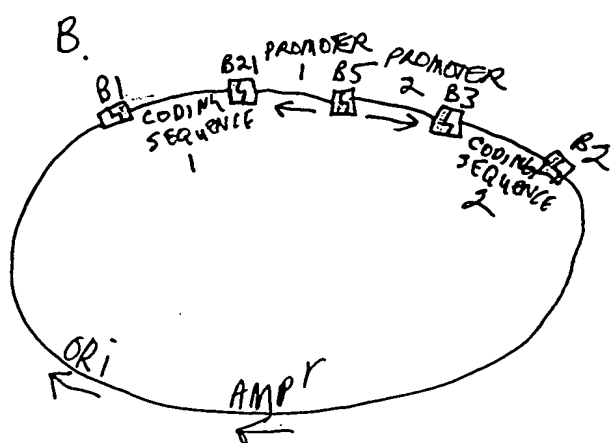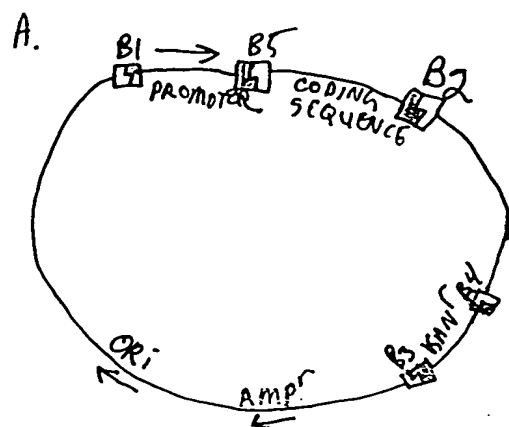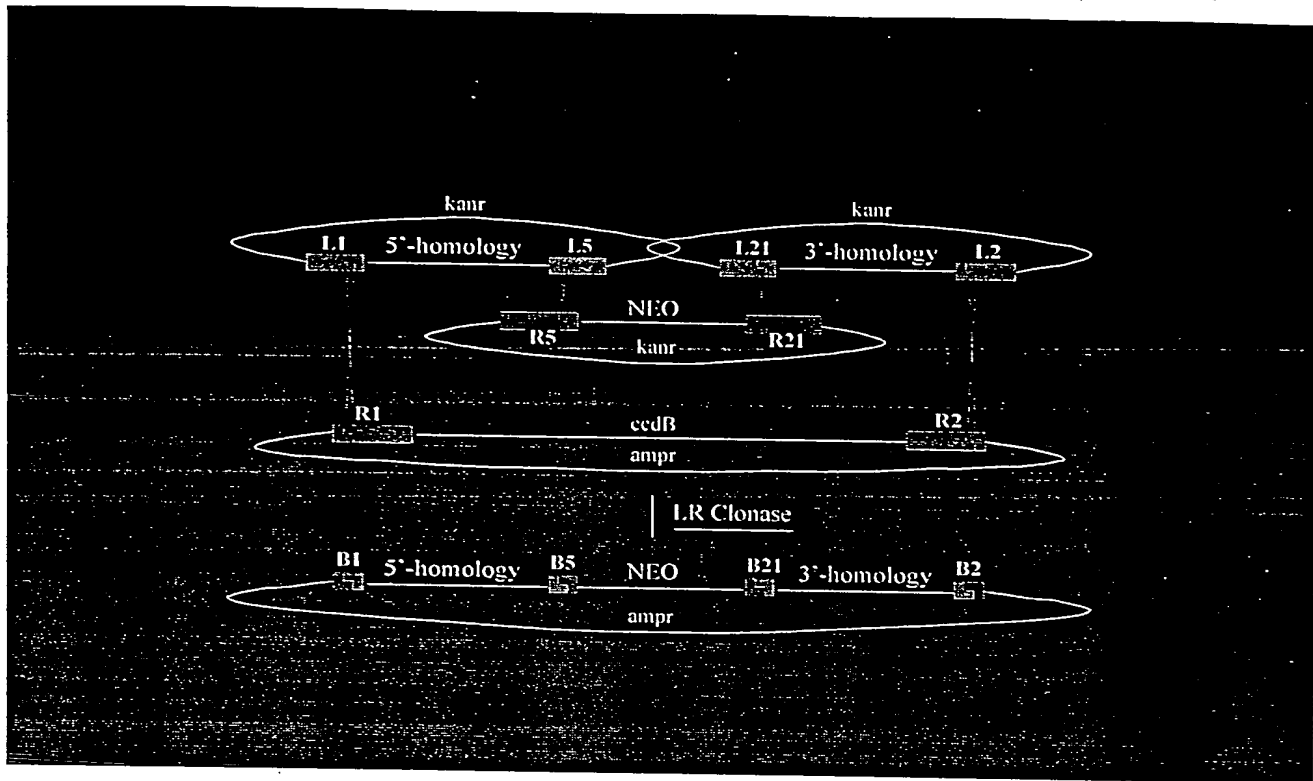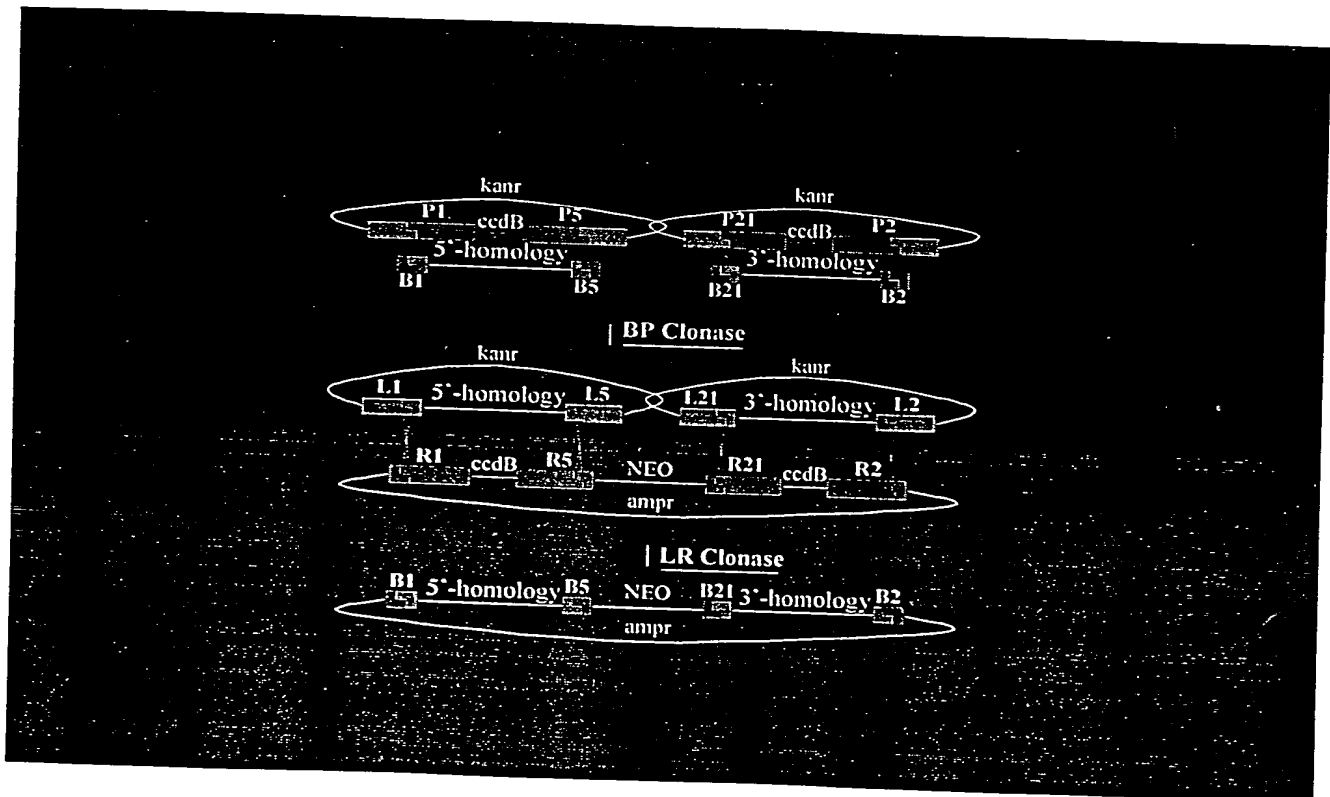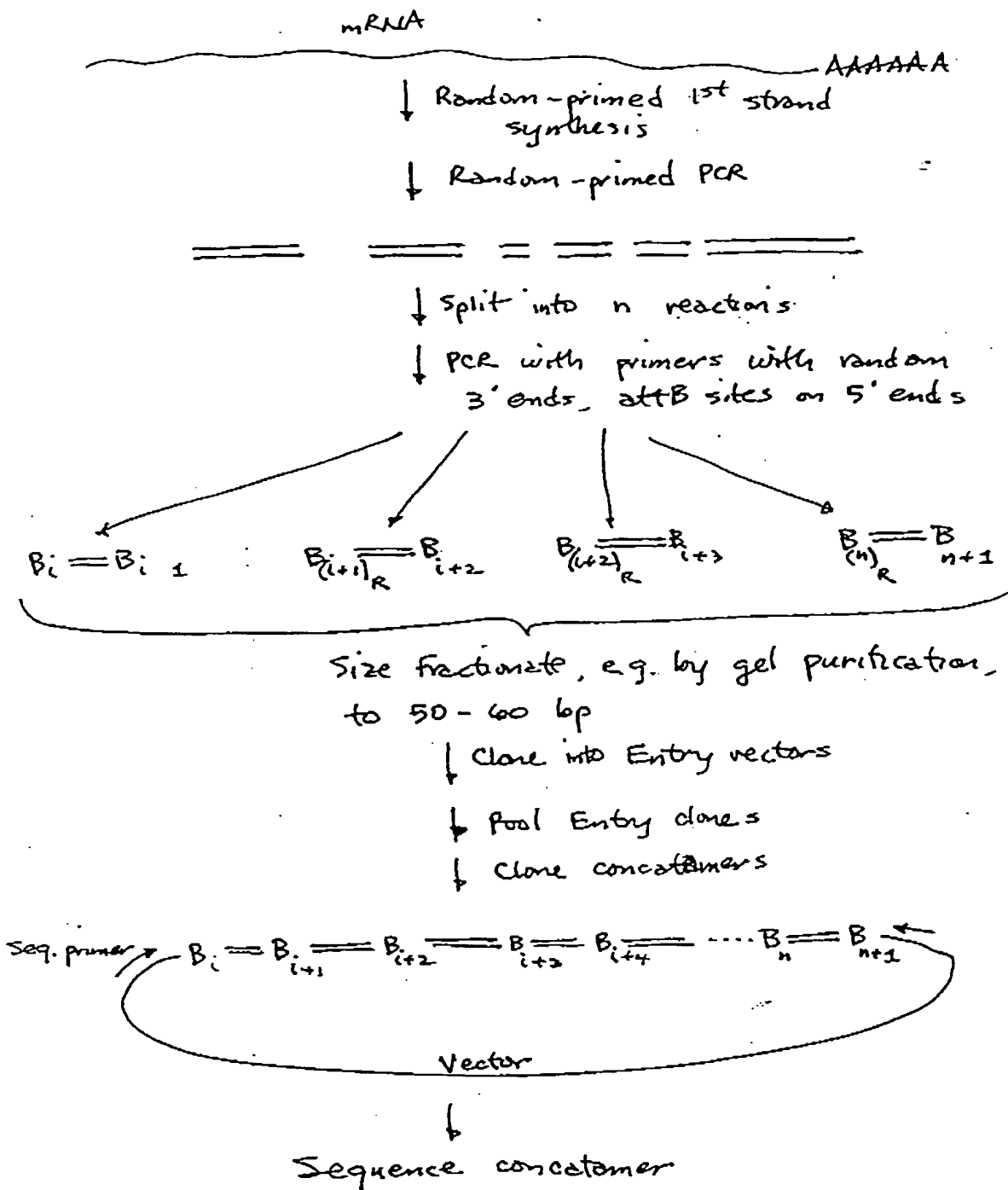
FIGURE 24A

attB0	AGCCTGCTTT**TTTATAC**TAACTTGAGC TCGGACGAAA**AAATATG**ATTGAACTCG	(SEQ ID NO:1)
attP0	GTTCAGCTTT**TTTATAC**TAAGTTGGCA CAAGTCGAAA**AAATATG**ATTCAACCGT	(SEQ ID NO:2)
attL0	AGCCTGCTTT**TTTATAC**TAAGTTGGCA TCGGACGAAA**AAATATG**ATTCAACCGT	(SEQ ID NO:3)
attR0	GTTCAGCTTT**TTTATAC**TAACTTGAGC CAAGTCGAAA**AAATATG**ATTGAACTCG	(SEQ ID NO:4)

attB1	AGCCTGCTTT**TTTGTAC**AAACTTGT TCGGACGAAA**AAATATG**TTTGAACA	(SEQ ID NO:5)
attP1	GTTCAGCTTT**TTTGTAC**AAAGTTGGCA CAAGTCGAAA**AAACATG**TTTCAACCGT	(SEQ ID NO:6)
attL1	AGCCTGCTTT**TTTGTAC**AAAGTTGGCA TCGGACGAAA**AAACATG**TTTCAACCGT	(SEQ ID NO:7)
attR1	GTTCAGCTTT**TTTGTAC**AAACTTGT CAAGTCGAAA**AAACATG**TTTGAACA	(SEQ ID NO:8)

attB2	ACCCAGCTTT**CTTGTAC**AAAGTGGT TGGGTCGAAA**GAATATG**TTTCACCA	(SEQ ID NO:9)
attP2	GTTCAGCTTT**CTTGTAC**AAAGTTGGCA CAAGTCGAAA**GAACATG**TTTCAACCGT	(SEQ ID NO:10)
attL2	ACCCAGCTTT**CTTGTAC**AAAGTTGGCA TGGGTCGAAA**GAACATG**TTTCAACCGT	(SEQ ID NO:11)
attR2	GTTCAGCTTT**CTTGTAC**AAAGTGGT CAAGTCGAAA**GAACATG**TTTGACCA	(SEQ ID NO:12)

attB5	CAACTTT**ATTATAC**AAAGTTGT GTTGAAA**TAATATG**TTTCAACA	(SEQ ID NO:13)
attP5	GTTCAACTTT**ATTATAC**AAAGTTGGCA CAAGTTGAAA**TAATATG**TTTCAACCGT	(SEQ ID NO:14)

FIGURE 24B

*att*L5	CAACTTT**ATTATAC**AAAGTTGGCA (SEQ ID NO:15)
	GTTGAAA**TAATATG**TTTCAACCGT
*att*R5	GTTCAACTTT**ATTATAC**AAAGTTGT (SEQ ID NO:16)
	CAAGTTGAAA**TAATATG**TTTCAACA

---

*att*B11	CAACTTT**TCTATAC**AAAGTTGT (SEQ ID NO:17)
	GTTGAAA**AGATATG**TTTCAACA
*att*P11	GTTCAACTTT**TCTATAC**AAAGTTGGCA (SEQ ID NO:18)
	CAAGTTGAAA**AGATATG**TTTCAACCGT
*att*L11	CAACTTT**TCTATAC**AAAGTTGGCA (SEQ ID NO:19)
	GTTGAAA**AGATATG**TTTCAACCGT
*att*R11	GTTCAACTTT**TCTATAC**AAAGTTGT (SEQ ID NO:20)
	CAAGTTGAAA**AGATATG**TTTCAACA

---

*att*B17	CAACTTT**TGTATAC**AAAGTTGT (SEQ ID NO:21)
	GTTGAAA**ACATATG**TTTCAACA
*att*P17	GTTCAACTTT**TGTATAC**AAAGTTGGCA (SEQ ID NO:22)
	CAAGTTGAAA**ACATATG**TTTCAACCGT
*att*L17	CAACTTT**TGTATAC**AAAGTTGGCA (SEQ ID NO:23)
	GTTGAAA**ACATATG**TTTCAACCGT
*att*R17	GTTCAACTTT**TGTATAC**AAAGTTGT (SEQ ID NO:24)
	CAAGTTGAAA**ACATATG**TTTCAACA

---

*att*B19	CAACTTT**TTCGTAC**AAAGTTGT (SEQ ID NO:25)
	GTTGAAA**AAGCATG**TTTCAACA
*att*P19	GTTCAACTTT**TTCGTAC**AAAGTTGGCA (SEQ ID NO:26)
	CAAGTTGAAA**AAGCATG**TTTCAACCGT
*att*L19	CAACTTT**TTCGTAC**AAAGTTGGCA (SEQ ID NO:27)
	GTTGAAA**AAGCATG**TTTCAACCGT
*att*R19	GTTCAACTTT**TTCGTAC**AAAGTTGT (SEQ ID NO:28)
	CAAGTTGAAA**AAGCATG**TTTCAACA

FIGURE 24C

attB20   CAACTTT**TTGGTAC**AAAGTTGT (SEQ ID NO:29)
              GTTGAAA**AACCATG**TTTCAACA

attP20   GTTCAACTTT**TTGGTAC**AAAGTTGGCA (SEQ ID NO:30)
              CAAGTTGAAA**AACCATG**TTTCAACCGT

attL20   CAACTTT**TTGGTAC**AAAGTTGGCA (SEQ ID NO:31)
              GTTGAAA**AACCATG**TTTCAACCGT

attR20   GTTCAACTTT**TTGGTAC**AAAGTTGT (SEQ ID NO:32)
              CAAGTTGAAA**AACCATG**TTTCAACA

---

attB21   CAACTTT**TTAATAC**AAAGTTGT (SEQ ID NO:33)
              GTTGAAA**AATTATG**TTTCAACA

attP21   GTTCAACTTT**TTAATAC**AAAGTTGGCA (SEQ ID NO:34)
              CAAGTTGAAA**AATTATG**TTTCAACCGT

attL21   CAACTTT**TTAATAC**AAAGTTGGCA (SEQ ID NO:35)
              GTTGAAA**AATTATG**TTTCAACCGT

attR21   GTTCAACTTT**TTAATAC**AAAGTTGT (SEQ ID NO:36)
              CAAGTTGAAA**AATTATG**TTTCAACA

FIGURE 25A

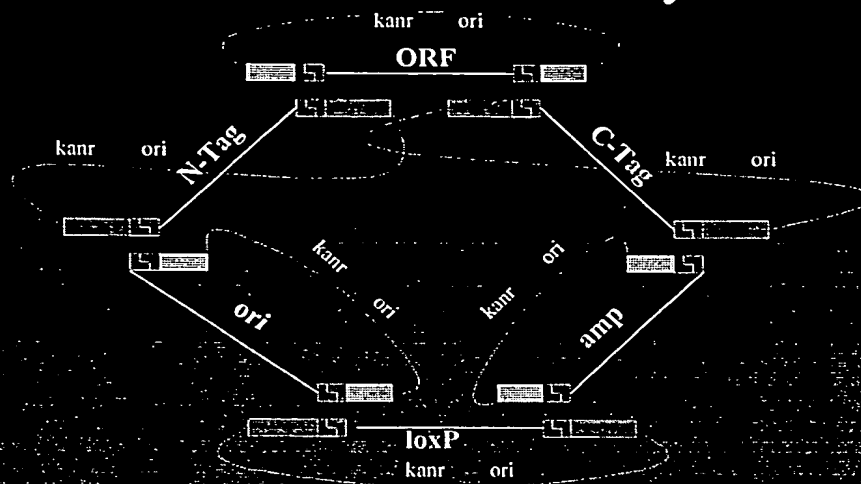Vector Assembly Using
Modular Vector Element Entry Clones

FIGURE 25B

Vector Assembly Using
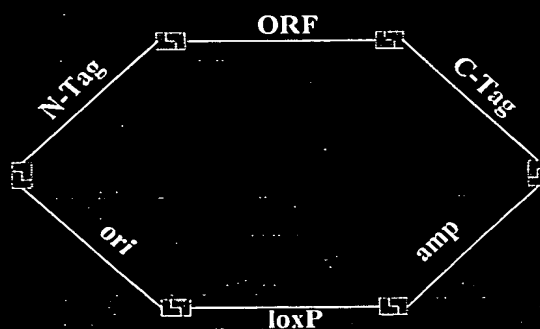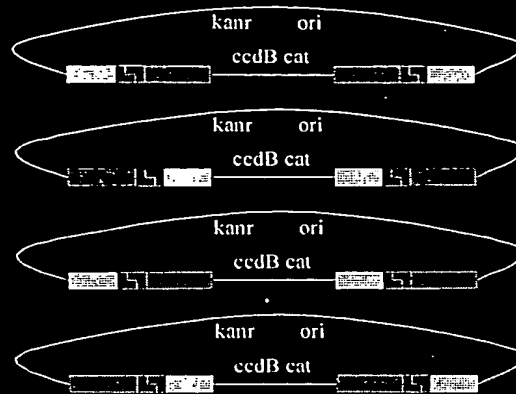Modular Vector Element Entry Clones

Construction of attP Plasmids
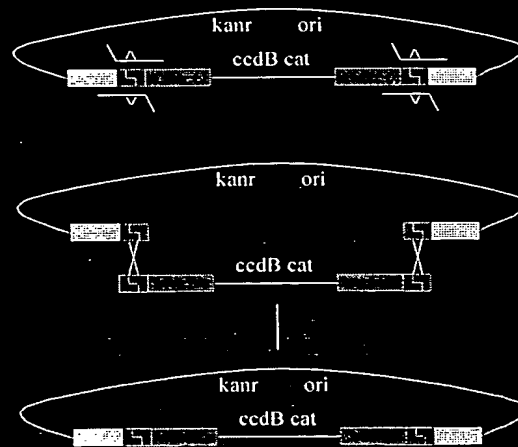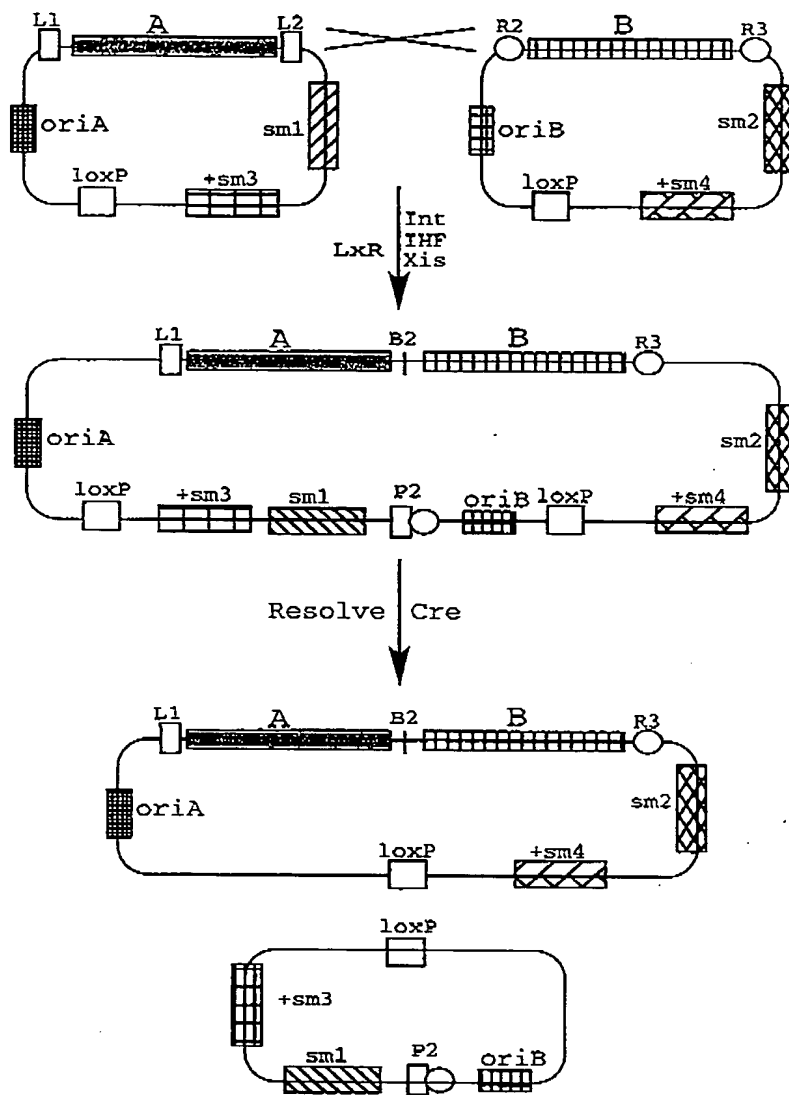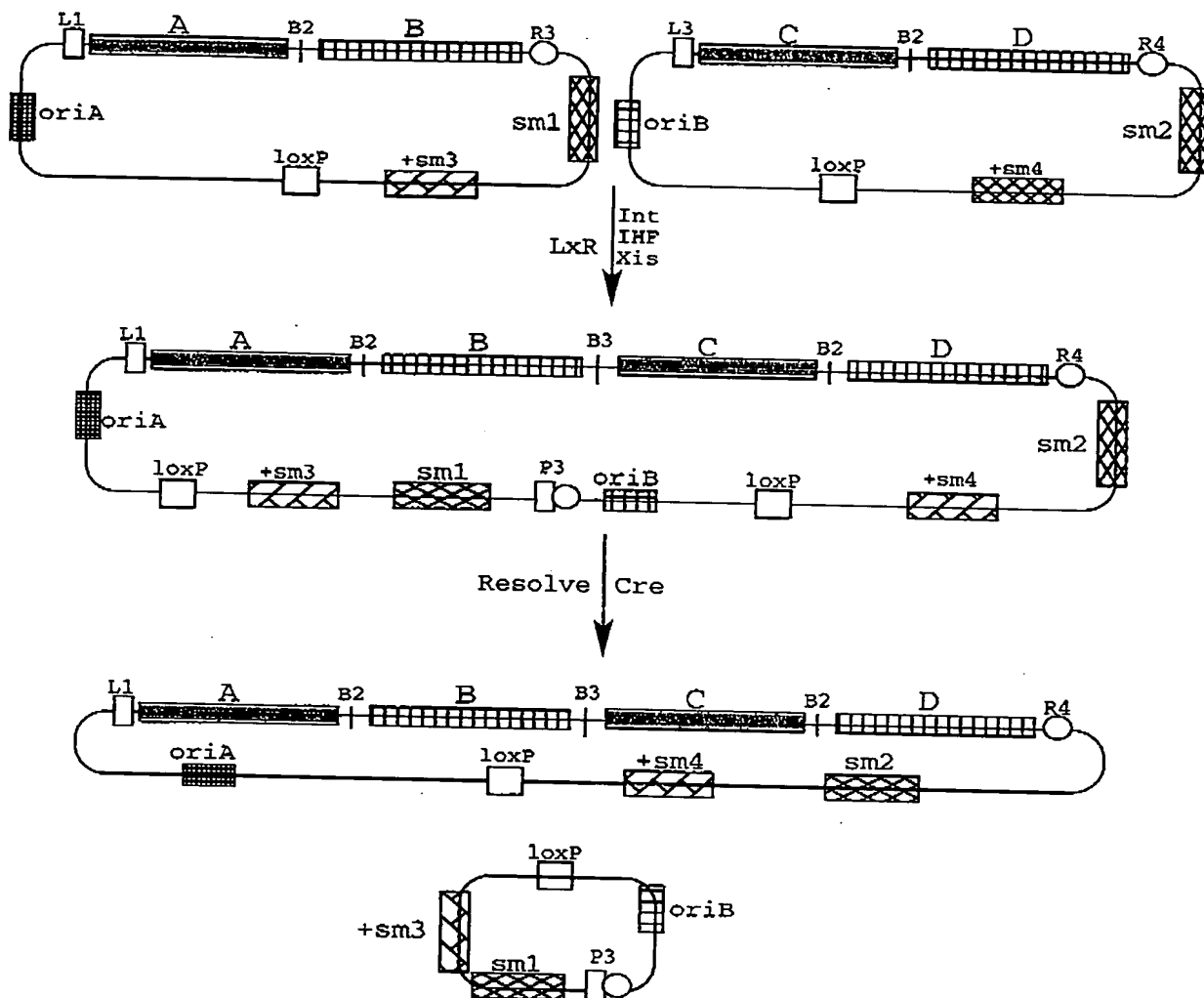
Construction of attP Plasmids

Transform host that will support replication of oriA but not oriB and moreover, is sensitive to +sm3 but resistant to +sm4.

FIGURE 27A

Transform host that will support replication of oriA but not oriB and moreover, is sensitive to +sm3 but resistant to +sm4.

FIGURE 27B